Electrochemically Modulated Liquid Chromatography

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Introduction

Liquid chromatographic separations rely on subtle differences in the interactions of analytes between a mobile phase and a stationary phase. Examples include the separation of charged species using ion-exchange chromatography, enantiomeric compounds via chiral selective chromatography, and polymeric materials by size-exclusion chromatography. Each of these separations, however, requires a different stationary phase. Thus, a large number of such phases and mobile phase elution schemes are required to tackle the diversity of the separations routinely encountered in the analytical laboratory.

Recently, a technique has been developed that begins to address the need for a large number of stationary phases. The approach, which is termed 'electrochemically modulated liquid chromatography' (EMLC), is based on the transformation of a conductive stationary phase in a conventional liquid chromatographic system into the working electrode of a three-electrode electrochemical cell. With this arrangement, the column packing serves both as a stationary phase and as a working electrode. This dual-purpose function provides the ability to induce changes in the interfacial properties (e.g. donor–acceptor strength, solvophobicity, and oxidation state) of conductive stationary phases through alterations in applied potential which subsequently translate into changes in analyte retention. The column can therefore be viewed as a compositionally tunable stationary phase with retention characteristics that can be manipulated both prior to as well as during elution by changes in applied potential. The latter capability presents an approach whereby improvements in a separation can be realized through a dynamic alteration in the composition of a stationary phase that is conceptually, but not mechanistically, analogous to conventional solvent gradient elution techniques in liquid chromatography or to temperature-programming strategies in gas chromatography. With electrochemically modulated liquid chromatography, however, the changes in retention reflect a temporal gradient in the composition of a stationary phase.

The following discusses the emergence of this new separation strategy as a tool in the arsenal of liquid chromatography techniques. After examining issues concerning the design and construction of the column, processes to control retention, illustrative applications demonstrating the versatility of the technique and issues related to improvements in column performance are presented.

Hardware

Column Development

The development of electrochemically modulated liquid chromatography can be traced back to the early 1950s and 1960s when concepts for the desalination of water and for the separation of metal ions explored a union of liquid chromatographic and electrochemical techniques. These and many subsequent investigations suffered from poor chromatographic efficiency (10–200 plates m^{-1}). As a consequence, interest in this technique has been limited until a few years ago. This limitation reflected the complexity in merging the design criteria necessary for both effective liquid chromatographic and electrochemical performance. In the case of the latter, a column must incorporate a reference electrode as a means to control precisely the potential applied to the stationary phase, and an auxiliary electrode as a means to complete the electrical circuit for current flow. In addition, the components of the column must be electrochemically as well as chemically inert and the mobile phase may need to be modified by the addition of an inert electrolyte to achieve the necessary solution conductivity; this modification is required to minimize the solution resistance of the mobile phase which, if not effectively manipulated, may degrade the ability to control the potential applied to the stationary phase.

Maintaining control over the applied potential is challenged, however, by the need for conductive stationary phases that have the characteristic size, shape, and surface area (e.g., 200 $m^2 g^{-1}$) required for high separation efficiencies with packed columns. A critical design attribute for high performance chromatography is a large surface area to solution volume ratio for the stationary phase with respect to the mobile phase. As a consequence, the small voids between the particles and within the pores of the particles that often make up a stationary phase appear as highly resistive pathways in an equivalent electrical circuit model for a column under potential control. These high-resistance networks define the time required to charge the electrical double layer at the surface of the stationary phase, and therefore the

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time needed for the column to equilibrate between changes in applied potential. This process is analogous to the time for a conventional liquid chromatographic column to equilibrate after a change in the composition of the mobile phase. Overall, a column must be designed to merge the demands of two methodologies that are in direct opposition in terms of their design specifications for effective performance.

A schematic diagram for a column that addresses the many design features requisite for the effective coupling of liquid chromatography and electrochemistry is shown in **Figure 1**. The design uses a ion-exchange membrane (e.g. Nafion®) as a tubular insert into a porous stainless-steel cylinder that forms the main body of the column. The porous stainless-steel cylinder functions both as a large surface area auxiliary electrode that can carry the large current

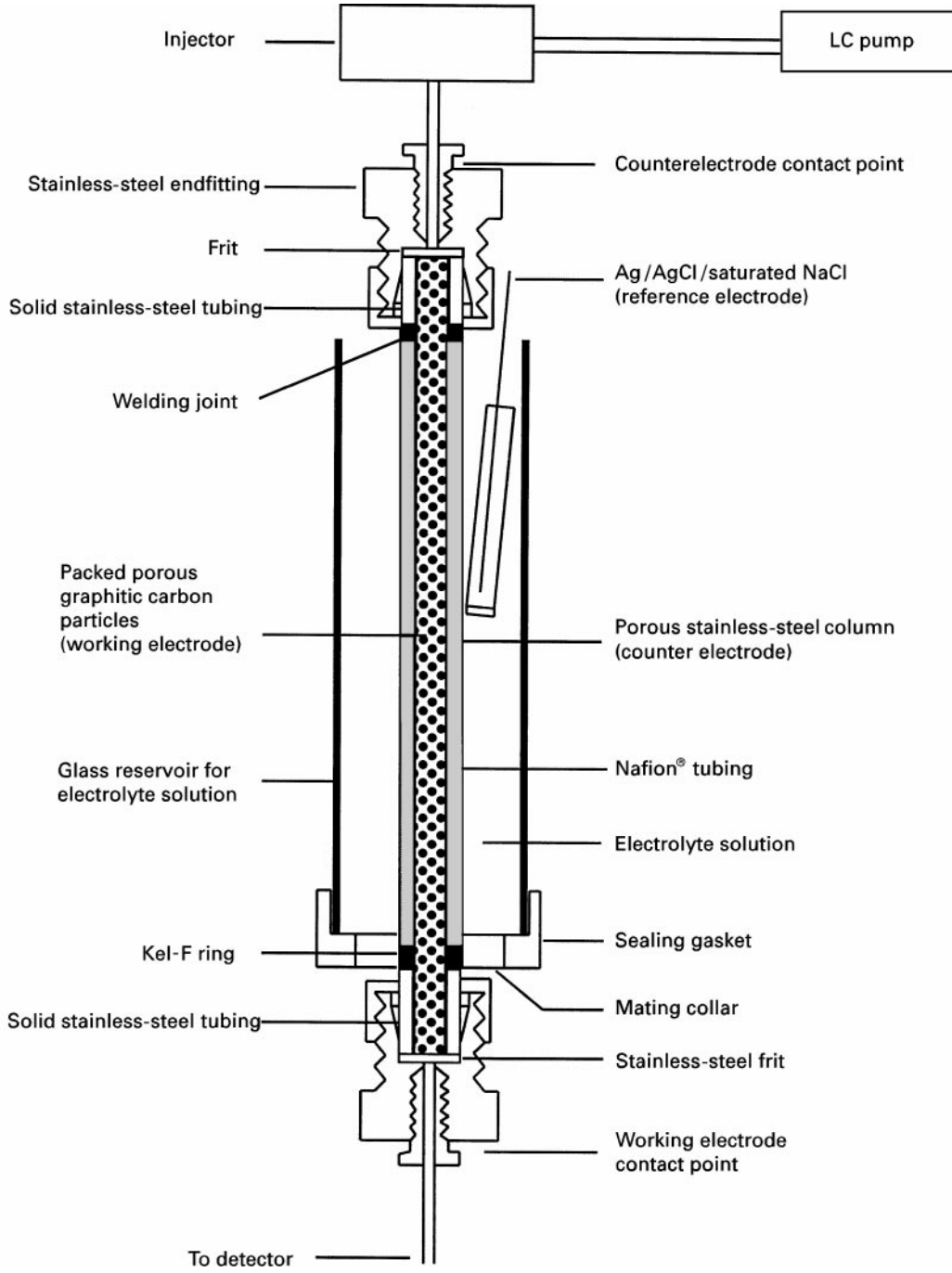

Figure 1 A schematic diagram of a column for electrochemically modulated liquid chromatography. A packing of porous graphitic carbon particles is shown only for illustrative purposes.

flow generated at the high surface area working electrode and as an inflexible container that defines the length and diameter of the column. The electrical circuit is completed by placing a reference electrode (e.g. Ag/AgCl(saturated NaCl)) inside a glass reservoir that surrounds the porous stainless-steel tubing and is filled with an aqueous electrolyte.

The ion-exchange membrane has three important functions in this design. It serves as:

- an insulating container that separates the stationary phase from the stainless-steel auxiliary electrode;
- an ionic bridge that completes the electrical connection between the stationary phase and auxiliary electrode; and
- a barrier that minimizes the loss of analyte through the porous stainless-steel cylinder as well as the possible contamination of the column by any electrolysis products generated at the auxiliary electrode.

With this design, the column equilibrates to alterations in applied potential on a time scale similar to that required for changes in mobile phase ($\sim$20 min), and performs at high chromatographic efficiencies (e.g., 15 000 plates m^{-1}).

Stationary Phases

Several types of conductive materials have been tested for use as packings in electrochemically modulated liquid chromatography. Some of the earlier examples investigated the utility of silver grains, mercury-coated platinum, uncoated graphitic particles, and various organic materials (e.g., conductive polymers) coated on graphitic particles. In general, the stationary phase material must meet the requirements in place for conventional liquid chromatography. These attributes include chemical inertness, high mechanical strength, high surface area, and small uniform size. To be effective as an electrode material, requirements including reasonable electrical conductivity ($\geq$0.01 Ω-cm) and stability over a wide range of applied voltage must be added to the list of attributes. For packings designed to undergo a formal change in oxidation state through an externally triggered electron-transfer process, the material must also be stable in both its oxidized and reduced forms.

Of the above materials, the greatest degree of success has been achieved to date using uncoated carbonaceous materials such as nonporous glassy carbon and porous graphitic carbon. These materials are stable both chemically (i.e. can be used in strongly acidic and basic mobile phases) and electrochemically (i.e. can be used over a wide range of applied potentials), have a high mechanical strength, and are commercially available as micron-sized particles. The non-porous material, has a lower surface area ($\sim$1 $m^2\,g^{-1}$) and therefore a lower separation efficiency, whereas the porous material has a higher surface area ($\sim$200 $m^2\,g^{-1}$) and therefore a higher separation efficiency. The coated materials, especially those that undergo a formal change in oxidation state (see below), potentially offer an additional dimension in affecting a separation, but complications regarding stability and reliable coating procedures remain significant issues.

Ancillary Instrumentation

Other than the features described for the column, this technique uses standard analytical laboratory hardware. Commercially available liquid chromatographic systems (e.g. pump, injector, and detector) and electrochemical hardware (e.g. potentiostat) are all that are needed to perform separation using electrochemically modulated liquid chromatography. To minimize the time required to re-equilibrate the column to changes, the use of a potentiostat with a high output power (i.e. a few watts) is recommended.

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Retention Concepts

Conventional liquid chromatographic separations rely on a partitioning of an analyte between a mobile phase and stationary phase. This equilibrium is generally expressed as a distribution coefficient (K_d) where:

$$K_d = C_s/C_m \qquad [1]$$

and C_s and C_m are the concentrations of an analyte in the stationary and mobile phases, respectively. Electrochemically modulated liquid chromatography therefore relies on the impact of the applied potential on the retention of an analyte through alterations in K_d.

Four principal formats have been used. The first format deals with an electrochemically induced reduction of metal ions (M^{n+}) onto a conductive stationary phase as M^0. The generic form of this reaction can be written as:

$$M^{n+} + ne^- \rightarrow M^0 \qquad [2]$$

where n represents the electron (e^-) stoichiometry of the reaction. The concentrations of the two species are then defined by the difference in the

standard reduction potential for the reaction (E^0) and the applied potential (E_{app}) through the Nernst equation:

$$E_{app} = E^0 + (0.059/n)(\log[M^0]/[M^{n+}]) \qquad [3]$$

Substituting M^0 and M^{n+} into eqn [1] for C_s and C_m, respectively, and then combining eqns [1] and [3] yields:

$$\log K_d = (n/0.059)(E^0 - E_{app}) \qquad [4]$$

Eqn [4] shows that K_d changes by a factor of ten for every $0.059/n$ V alteration of the applied potential. However, while providing a means for the quantitative deposition of metal ions, the process suffers from poor selectivity. That is, metal ions with standard reduction potentials more positive than the applied potential are exhaustively deposited onto the column, whereas those with standard reduction potentials more negative than the applied potential pass through the column without being retained. Strategies that have been devised to address this limitation have been studied in a few specific applications.

The second format takes a notably different tactic in that the potential applied to a graphitic stationary phase is used to manipulate the chemical composition of a mobile phase. This strategy relies on the oxidation or reduction of water either to increase or decrease, respectively, the acidity of the mobile phase. This general tactic, though explored only briefly to manipulate the pH-dependent binding of metal ions to chelates immobilized onto the column packing, will undoubtedly be more extensively exploited as interest in this new separation technique increases.

The third format, which has been the most used of the four, utilizes uncoated graphitic stationary phases. The basis of this format derives largely from the effect of the applied potential on the donor–acceptor properties (e.g. electrostatic and dipolar interactions) of the packing and is illustrated in **Figure 2**. Thus, as the applied potential becomes more positive, the carbonaceous packing becomes a stronger acceptor and analytes with a predominant donor character are more strongly retained. The retention of analytes with a predominant acceptor character, in contrast, increases as the applied potential becomes more negative. This approach has been employed, as discussed in the following section, to separate a wide ranges of samples, including, charged and neutral aromatic compounds, important pharmaceutical agents, and enantiomeric pairs.

The fourth format takes advantage of a different form of electrochemically modulated separation in which the composition of a column packed with an electroactive material is transformed by its oxidation/reduction. Eqn [5] depicts this process whereby a coating (C) is switched between its reduced and oxidized forms with the concomitant expulsion or uptake of anions (A^-). The analogous reaction:

$$C^+A^- + e^- = C^0 + A^- \qquad [5]$$

can be written for coatings that involve cations as counterions. Thus, the charge density of the ion-exchange sites on a stationary phase can be tuned

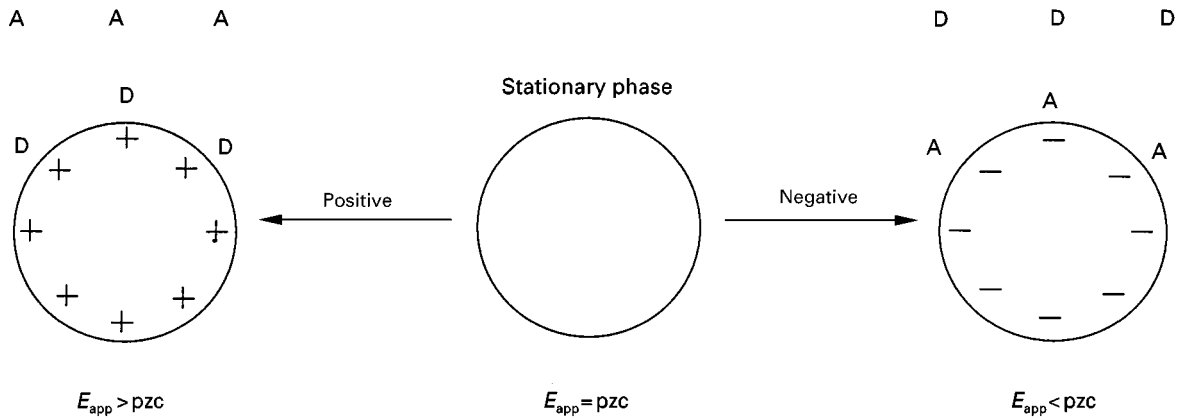


Figure 2 Idealized representations of the interactions of donor (D) and acceptor (A) analytes with a conductive stationary phase as a function of applied potential (E_{app}). Collectively, changes in applied potential alter the extent of the interaction of such analytes through a change in the surface charge of the stationary phase. At applied potential greater than the potential of zero charge (pzc), the stationary phase has a net positive surface charge and therefore a greater acceptor strength. This increase results in an increase in the attractive interaction between the stationary phase and a donor analyte. The converse applies as the applied potential becomes more negative than the pzc. In this case, the donor strength of the stationary phase increases, and the attractive interaction between the stationary phase and an acceptor analyte increases.

through changes in applied potential. Several intriguing features of this strategy have been described.

Electrochemically modulated liquid chromatography, therefore, presents a broad range of tactics that can, as demonstrated in the next section, be exploited in tackling the many separation challenges faced in the analytical laboratory.

Applications

This section presents a few examples of the capabilities of the technique, including the separation of aromatic compounds, pharmaceutical agents, and chiral compounds. The use in a concentration format is also described.

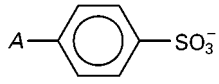
Aromatic Sulfonates

The earliest demonstration of the ability of electrochemically modulated liquid chromatography to separation complex mixtures at performance levels that rivaled conventional liquid chromatographic separations used porous graphitic carbon as the column packing and the aromatic sulfonates in **Table 1** as analytes. Examples are presented in **Figure 3**. These results show that: (1) the retention for all of the analytes increases as the applied potential moves positive, (2) the retention of all the analytes (**Figure 4**) exhibit a linear dependence on applied potential, (3) the magnitude of the change in retention differs between analytes, and (4) all six analytes in the mixtue are baseline resolved at $+0.5$ V. These results confirm that the analytical figures of merit (e.g. resolution and retention time) can be readily manipulated by changes in applied potential, and that the change in the surface charge of the stationary phase plays a key role in altering the extent of retention.

It is also possible to affect such separations by manipulating the applied potential during analyte elution, a strategy that parallels temperature programming in gas chromatography. **Figure 5** demonstrates this possibility whereby a linear voltage ramp is imposed upon the stationary phase from $+0.3$ V to -1.0 V immediately upon sample injection. This strategy increases the resolution of the compounds that eluted early at the open circuit potential (i.e. the condition used in conventional liquid chromatography) by increasing their retention. At the same time, this waveform decreases the retention and narrows the bands for those compounds eluting much later in the open circuit separation. There is also a notable decrease in the time required to complete the separation ($\sim$4 min). Thus, a stationary phase gradient offers a pathway for the optimization of separations.

Table 1 Chemical structures and labelling schemes of the aromatic sulfonate derivatives

Compound	*Acronym*	*A*
1	ABS	NH_2
2	HBS	OH
3	BS	H
4	TBS	CH_3
5	CBS	Cl
6	BAS	COOH



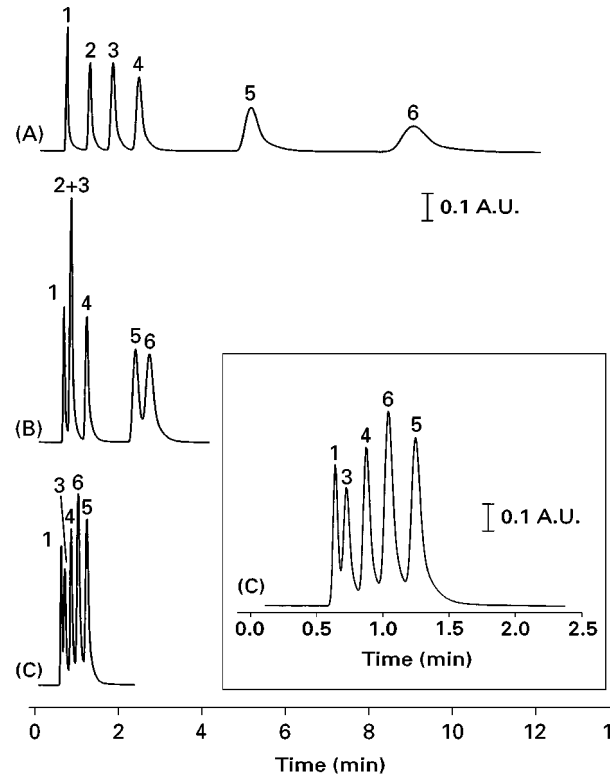
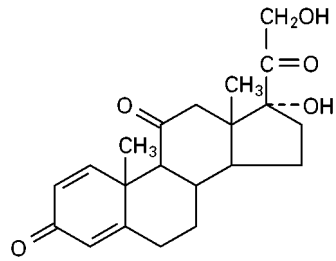


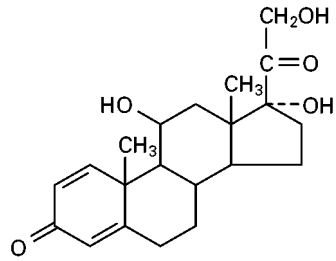
Figure 3 Separations of a mixture of aromatic sulfonates (see Table 1) using a porous graphitic carbon stationary phase at $+0.50$ V (A), open circuit (B), and -0.60 V (C). The mobile phase was water (0.1 M $LiClO_4$, 0.1% TFA) and acetonitrile (0.1 M $LiClO_4$) (88 : 12, v/v). The flow rate was 0.90 mL min^{-1}. (Reproduced with permission from Ting EY and Porter MD (1998) *Analytical Chemistry* 70: 94–99. Copyright ACS Publications.)

Corticosteroids

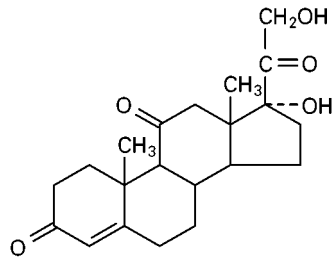
Separations of structurally similar compounds can readily be fine tuned using electrochemically modulated liquid chromatography. The challenge presented by the separation of four corticosteroids, [I]–[IV], at a porous graphitic carbon stationary phase is a particularly intriguing example.



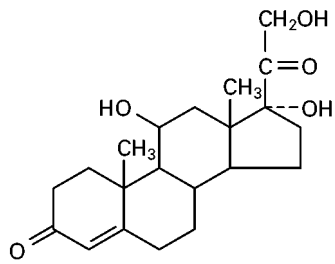
Prednisone [I]



Prednisolone [II]



Cortisone [III]



Hydrocortisone [IV]

The complication with this mixture is the discrimination between structures containing differing numbers of double bonds (e.g. prednisone vs. cortisone) and/or small differences in the identity and number of substituents (e.g. cortisone vs. hydrocortisone). On bonded reversed phases, compounds with subtle differences in functionality generally exhibit greater differences in retention than those that differ by the number of double bonds. The converse is often more important for separations on porous graphitic carbon, which possesses π-electron sensitivity superimposed upon reversed phase characteristics. Thus,

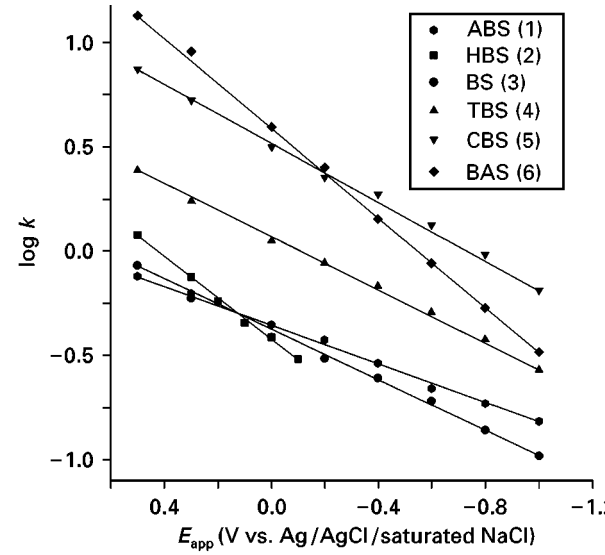


Figure 4 Plots of log k vs. applied potential (E_{app}) for the separations of the mixture of aromatic sulfonates (see Table 1) between $+0.50$ V and -1.00 V. Note that the values of HBS extend only from $+0.50$ V to -0.10 V because of its electroreduction which occurs at an E_{app} between -0.10 and -0.20 V. (Reproduced with permission from Ting EY and Porter MD (1998) *Analytical Chemistry* 70: 94–99. Copyright ACS Publications.)

strategies that would enhance the sensitivity of carbonaceous materials toward substituent differences, would improve their applicability as packings especially as they are more stable than many of the bonded phases when exposed to strongly acidic and strongly alkaline solutions.

The chromatograms in **Figure 6** present the separations of the four compounds as a function of applied potential using a porous graphitic carbon stationary phase. At the open circuit potential, the separation is only marginally effective in resolving the four compounds. Changes in the applied potential, however, have a marked influence on the separation. Interestingly, the retention of prednisone and prednisolone increase as the applied potential becomes more negative, with that for prednisone becoming greater than that for prednisolone. This difference transposes the elution order for the two compounds. The same general dependencies for the retention of cortisone and hydrocortisone are observed. In contrast to the aromatic sulfonates, the supporting electrolyte can compete in some instances with the analyte for binding sites on the stationary phase, leading to retention dependencies that are nonlinear with respect to changes in applied potential. As a result of the combined effect of these dependencies, all four components are fully resolved at -700 mV, with a total elution time of $\sim$10 min. Thus, separations based on manipulating the applied potential provide an effective and

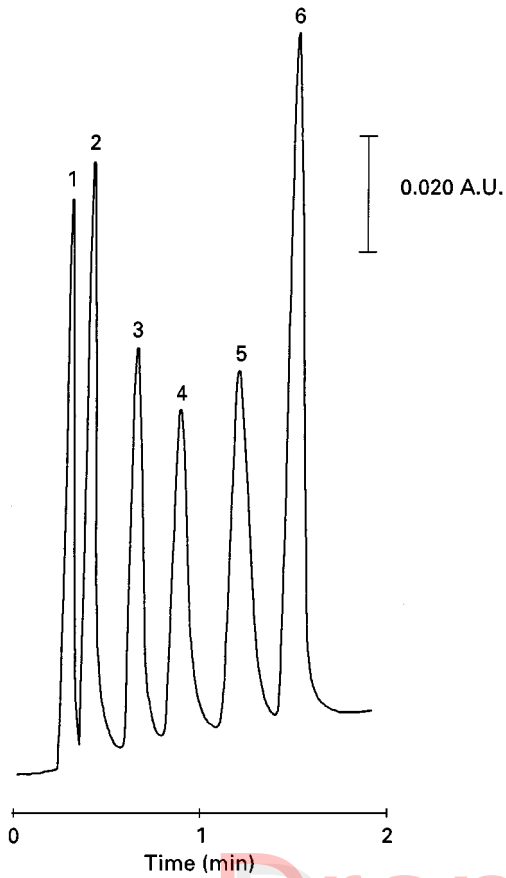


Figure 5 Separation of the six-component mixture of aromatic sulfonates (see Table 1) on a porous graphitic carbon stationary phase obtained by applying a linear voltage ramp from + 0.30 V to − 1.00 V. The voltage ramp was initiated immediately upon sample injection at 10 mV s^{-1}. The mobile phase consisted of aqueous 0.1 M $LiClO_4$ with 1.5% acetonitrile. The flow rate was 0.75 mL min^{-1} and the analyte concentrations were ~ 200 ppm. The shift in the baseline reflects the gradual decrease in the interfacial concentration of the ClO_4^- anion of the supporting electrolyte in the mobile phase as the applied potential becomes more negative. The background baseline gradually returns to its initial value in ~ 10 min. (Reproduced with permission from Deinhamer RS, Ting E and Porter MD (1993) *Journal of Electroanalytical Chemistry* 362 : 295–299. Copyright Elsevier Science SA.)

facile means to tackle the challenges posed by the strong structural similarity of these compounds.

Chiral Compounds

This technique can also be applied to separations of racemic mixtures. These separations, usually accomplished with a chiral selector either immobilized onto a stationary phase or as an additive to a mobile phase, are critical to pharmaceutical areas because therapeutic efficacy is often enantiomerically specific. However, the high level of structural discrimination required to separate optical isomers dictates the use of a variety of stationary-mobile phase combinations. This separation can be realized by the reversible electrosorption of a chiral compound added to the mobile phase, a process that converts an achiral column into a chiral column. The concept is illustrated in **Figure 7**, using β-cyclodextrin as the chiral mobile phase additive. The choice of this additive reflects the notable enhancement of the interactions between hydroxyl functionalities and a carbonaceous packing as the applied potential becomes increasingly positive.

Results are presented in **Figure 8** for the separations with and without the chiral selector present as a mobile phase additive at several different applied

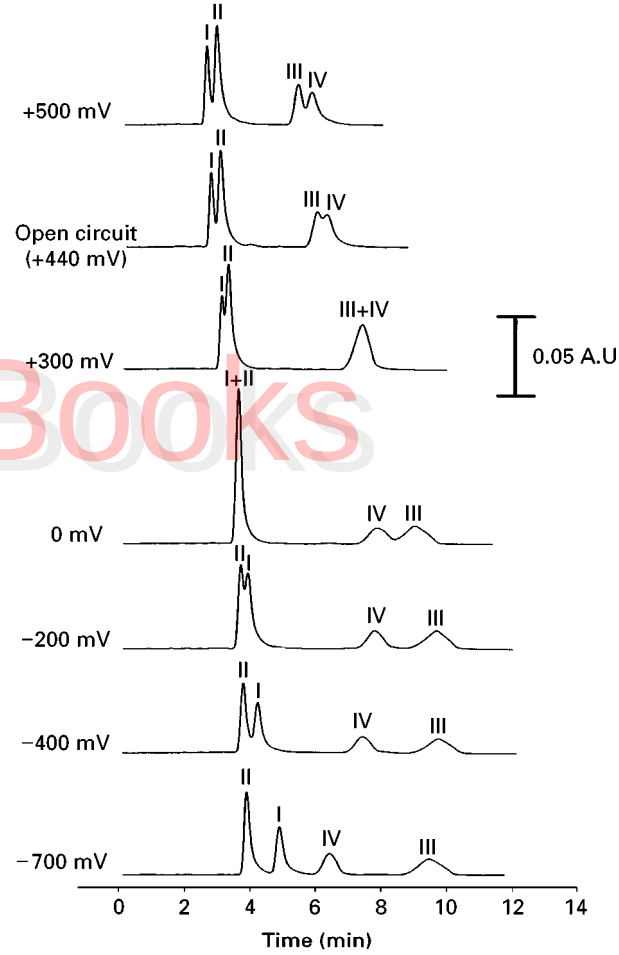


Figure 6 Separations using EMLC of a mixture of the mixture of prednisone [I], prednisolone [II], cortisone [III], and hydrocortisone [IV] at a porous graphite carbon stationary phase as a function of applied voltage: + 500 mV, open circuit (i.e. + 440 mV), + 300 mV, 0 mV, − 200 mV, − 400 mV, − 700 mV. All applied voltages are given with respect to an Ag/AgCl (saturated NaCl) electrode. The mobile phase was composed of two components: water (0.1 M $HClO_4$)/acetonitrile (0.1 M $LiClO_4$) 50 : 50 (v/v). The flow rate was 0.90 mL min^{-1}. (Reproduced with permission from Ting EY and Porter MD (1997) *Analytical Chemistry* 69: 675–678. Copyright ACS Publications.)

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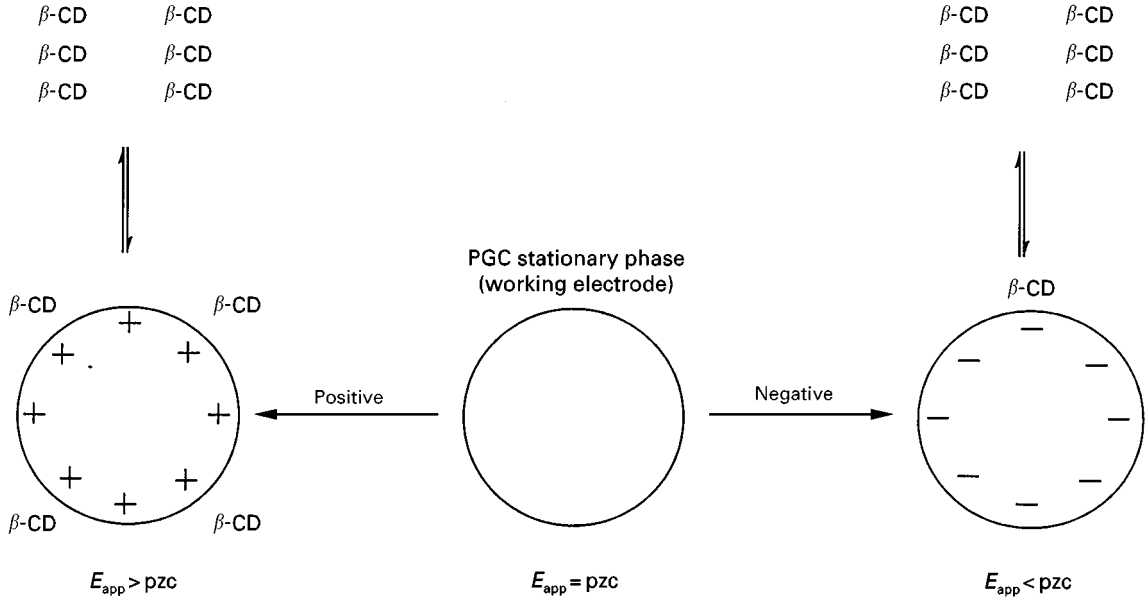

Figure 7 Idealized depiction of the electrosorption process of β-cyclodextrin (β-CD) onto porous graphite carbon (PGC). pzc, potential of zero charge.

potentials. The sedative hexobarbital is used as the chiral analyte. As expected for an achiral system, there is no detectable separation of enantiomers in Figure 8A. Furthermore, the retention of the sedative increases as the applied potential becomes more negative. This trend is consistent with the dominant acceptor character of this compound because of its protonation at the mobile phase (pH ~ 2).

Marked differences in the separations are found when β-cyclodextrin is added to the mobile phase. Figure 8B shows the results; the presence of the additive results in a separation of the hexobartital enantiomers at several applied potentials. Enantiomeric separations are evident at −1.0, 0.0, and +0.5 V, with the highest resolution at +0.5 V. There is no observable enantiomeric separation for −0.5 V. Experiments in which an excess of one enantiomer was added to the mixture reveal the enantiomeric separation is controlled at the more negative applied potentials by interactions with the chiral additive in the mobile phase, and at the more positive applied potentials by interactions with the electrosorbed chiral selector.

Experiments have also shown that the electrosorption-based modification of the porous graphitic carbon phase is reversible. This observation suggests that electrosorption may serve as a versatile means for altering the surface composition of conductive stationary phases whereby a chiral column for enantiomeric separations could, for example, be readily converted to a column with reversed phase character by the electrosorption of another mobile phase additive (e.g. a long alkyl chain alcohol). This intriguing strategy offers the possibility of requiring only one column for the separation of a wide range of complex mixtures.

Concentration

Another intriguing application of this technique is sample concentration. This approach takes advantage of the ability to modulate the capacity of a stationary phase, for example, by changes in the oxidation state of an electrochemically transformable stationary phase (e.g. eqn [5]). This strategy is similar to that employed in solid-phase extractions except that changes in applied potential rather than changes in solvent can be used to concentrate and then strip the analyte from the column.

An example of this strategy is the concentration of the dansylated amino acid tryptophan using a nonporous carbon stationary phase that is coated with a thin film of polypyrrole. Polypyrrole, which can be polymerized electrochemically onto a wide range of electrode materials, can be switched between its oxidized and reduced forms with the concomitant uptake or expulsion of anions, as represented in **Figure 9**. Thus, changes in applied potential transform the stationary phase between one devoid of ion-exchange sites to one with a large number of exchange sites. This transformation is evident in the cyclic voltammetric curve shown in **Figure 10**. This curve demonstrates the change of the coating from its neutrally charged (reduced) form at the more negative

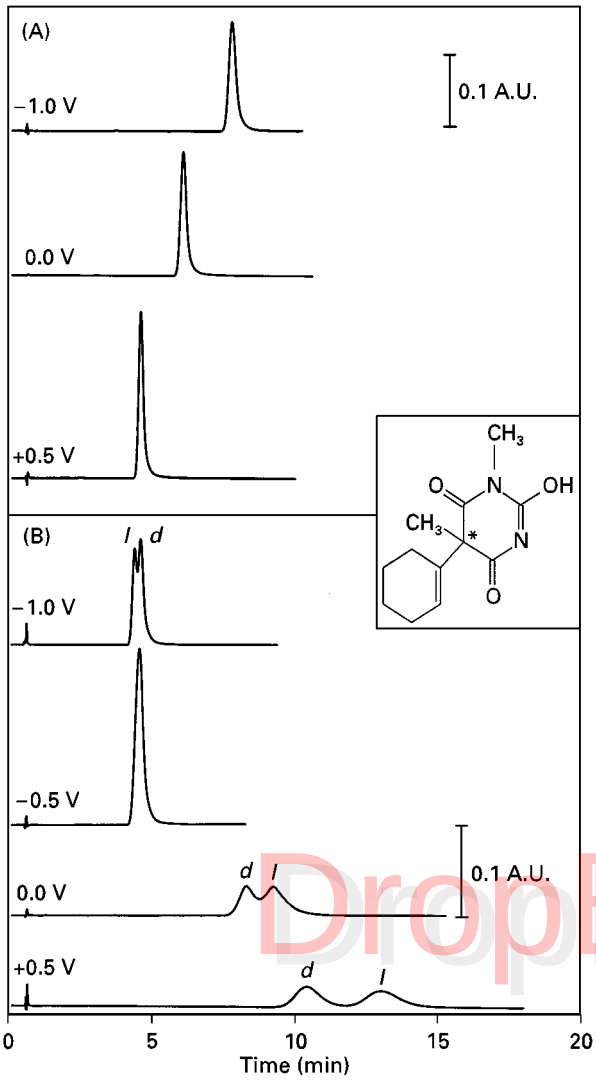

Figure 8 Separations of hexobarbital enantiomers (*d* and *l*), using a mobile phase of 20% acetonitrile and 80% water (0.1 M $LiClO_4$, 20 mM phosphate buffer (pH ~ 2)), a flow rate 0.9 mL min^{-1}, a detection wavelength of 220 nm, and an Ag/AgCl (saturated NaCl) reference electrode. (A) Separations in mobile phase devoid of β-cyclodextrin at −1.0 V, −0.5 V, 0.0 V and +0.5 V. (B) Separations with 15 mM β-cyclodextrin as a mobile phase additive at −1.0 V, −0.5 V, 0.0 V and +0.5 V. The concentration of hexobarbital is ~500 ppm, and the injection volume was 0.5 μL. (Reproduced with permission from Ho MK, Wang S and Porter MD (1998) *Analytical Chemistry* 70: 4314–4319. Copyright ACS Publications.)

values of applied potential to its cationic (oxidized) form as the applied potential becomes increasingly positive.

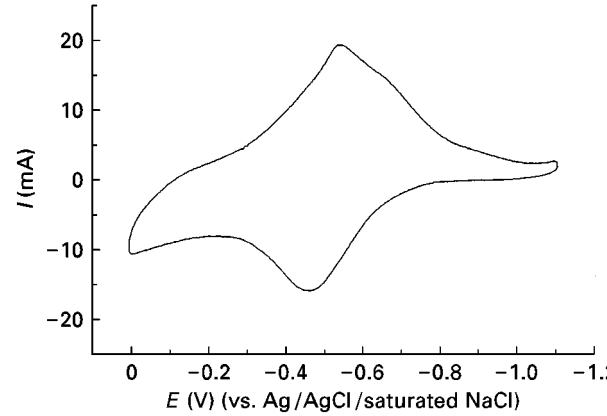

Figure 10 Cyclic voltammetric curve for a nonporous glassy carbon packing that has been coated with a thin film of polypyrrole in 0.10 M sodium benzoate. A silver wire is used as a quasi-reference electrode, and the scan rate was 5 mV s^{-1}. (Reproduced with permission from Deinhamer RS, Shimazu K and Porter MD (1995) *Journal of Electroanalytical Chemistry* 387: 35–46. Copyright Elsevier SA.)

Figure 11 presents the details of the experiment by showing the breakthrough curve for the concentration process. The experiment begins by passing a mobile phase containing dansylated tryptophan through the column continuously while the applied potential is held at −1.00 V. The applied potential is then stepped to and held at 0.00 V to oxidize the coating to its cationic form. Upon stepping potential to 0.00 V, the absorbance of the eluent rapidly decreases to the value of the acetate mobile phase, reflecting the exhaustive uptake of the amino acid by the coating. After ~67 min, the absorbance increases slowly to the value initially observed at −1.00 V.

This increase signals the saturation of the coating by the uptake of the amino acid from the highly dilute solution; the coating is fully saturated in 72.5 min. After saturation, the applied potential is stepped back to −1.00 V to reduce the coating to its neutral form, and strip the amino acid from the column in a narrow elution band. An analysis of the data gives a concentration factor of ~33 and a recovery of ~98%. Thus, this strategy offers an alternative approach to the solvent elution processes used in most other concentration techniques in that the stripping of an analyte from the column can be facilitated by a change in applied potential, opening the possibility of large reductions in the volumes of generated wastes.

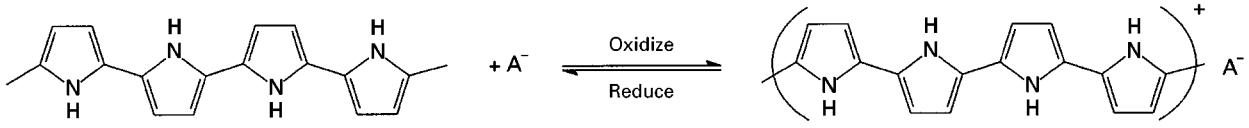

Figure 9 Electrochemical switching of polypyrrole between its oxidized and reduced forms showing the respective uptake or expulsion of an anion A^-.

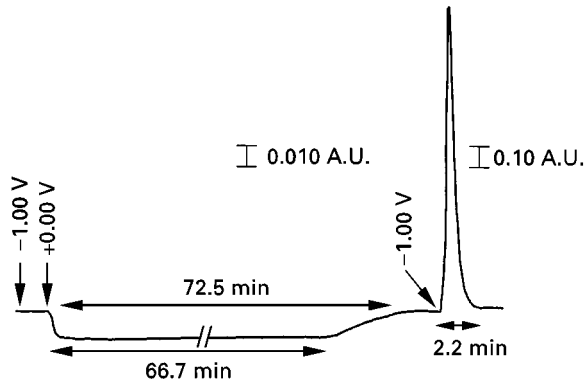

Figure 11 Breakthrough curve showing the concentration of a 1.6 ppm solution of dansylated tryptophan at a polypyrrole stationary phase. The applied potential was initially held at − 1.00 V, stepped to 0.00 V for 72.5 min, and then stepped back to − 1.00 V. The mobile phase consisted of 1.6 ppm dansylated amino acid tryptophan in 10 mM pH 6 acetate buffer. (Reproduced with permission from Deinhamer RS, Shimazu K and Porter MD (1995) *Journal of Electroanalytical Chemistry* 387: 35–46. Copyright Elsevier Science SA.)

Future Developments

Electrochemically modulated liquid chromatography is emerging as a valuable addition to the arsenal of liquid chromatographic techniques used in tacking the many challenges encountered in the day-to-day operations of the analytical laboratory. Advances in this area will continue with improvements in column design that reduce the equilibration time to changes in applied potential. Such developments will enhance the ability of the analyst to manipulate separations by dynamically altering the applied potential during analyte elution.

There are also several intriguing opportunities in the creation of separation formats using new types of stationary phases. Other types of conducting phases based, for example, on electrochemically transformable coatings offer pathways to manipulate separations by variations in the porosity of a coating which can be affected by the size of the dopant ion used in the electrochemical coating process. Variations in porosity may add size discrimination to the selectivity of the separation process. In addition, the incorporation of functional groups as substituents on the monomer precursors used for polymer formation can be exploited to design coating with hydrophobicity–hydrophilicity differences.

Another area of fruitful development lies in the coupling of this technique to other detector formats. Mass spectrometry, for instance, has proven invaluable as a detector in a wide range of chromatographic applications. The ability of electrochemically modulated liquid chromatography to address separations that require the use of gradient-elution strategies may overcome the associated problems of a continuing changing background when using electrospray mass spectrometry. Enhancements may also be realized by electrochemically transforming an analyte to a form that improves not only its separation but also its response to conventional optical detection formats. These developments, coupled with systematic explorations of the interactions operative in the many retention mechanisms, promise to stimulate the widespread interest in this new mode of liquid chromatographic separations.

See also: **III/Steroids:** Liquid Chromatography and Thin-Layer (Planar) Chromatography. **Chiral Separations:** Cyclodextrins and Other Inclusion Complexation Approaches; Liquid Chromatography.

Further Reading

Deinhammer RS, Ting EY and Porter MD (1993) Electrochemically modulated liquid chromatography (EMLC): a new approach to gradient elution separations. *Journal of Electroanalytical Chemistry* 362: 295–299.

Deinhammer RS, Porter MD and Shimazu K (1995) Retention characteristics of polypyrrole as a stationary phase for the electrochemically modulated liquid chromatographic (EMLC) separations of dansyl amino acids. *Journal of Electroanalytical Chemistry* 387: 35–46.

Fujinaga T and Kihara S (1977) Electrolytic chromatography and coulopotentiography – a rapid electrolysis at the column electrode used for the preparation, separation, concentration, and estimation of trace and/or unstable substances. *CRC Critical Reviews in Analytical Chemistry* 6: 223–253.

Ge H and Wallace GG (1989) Characterization of conducting polymeric stationary phases and electrochemically controlled high-performance liquid chromatography. *Analytical Chemistry* 61: 2391–2394.

Ge H, Teasdale PR and Wallace GG (1991) Electrochemical chromatography – packings, hardware, and mechanisms of interaction. *Journal of Chromatography* 544: 305–316.

Ghatak-Roy AR and Martin CR (1986) Electromodulated ion exchange chromatography. *Analytical Chemistry* 58: 1574–1575.

Hern JL and Strohl JH (1978) Modified graphites for chelation and ion exchange. *Analytical Chemistry* 50: 1955.

Ho MK, Wang S and Porter MD (1998) Electrosorption-based modification of porous graphitic carbon: use of electrochemically modulated liquid chromatography to create a chiral stationary phase for enantiomeric separations. *Analytical Chemistry* 70: 4314–4319.

Lam P, Elliker PR, Wnek GE and Przybycien TM (1995) Towards an electrochemically modulated chromatographic stationary phase. *Journal of Chromatography* 707: 29–33.

Nagaoka T, Fujimoto M, Nakao H *et al.* (1994) Electrochemical separation of ionic compounds using a conductive stationary phase coated with polyaniline or polypyrrole film, and ion exchange properties of conductive polymers. *Journal of Electroanalytical Chemistry* 364: 179–189.

Suzuki T, Noble RD and Koval CA (1997) Electrochemistry, stability, and alkene complexation chemistry for copper(I) triflate in aqueous solution. Potential for use in electrochemically modulated complexation-based separation processes. *Inorganic Chemistry* 36: 136–140.

Ting EY and Porter MD (1997) Separations of corticosteroids using electrochemically modulated liquid chromatography: selectivity enhancements at a porous graphitic carbon stationary phase. *Analytical Chemistry* 69: 675–678.

Ting EY and Porter MD (1998) Column design for electrochemically modulated liquid chromatography. *Analytical Chemistry* 70: 94–99.

Electrochromatography

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Introduction

High performance liquid chromatography (HPLC) is an established technique with a vast number of stationary phases available, but the efficiency is ultimately limited by the size of the particles used to pack the columns. Theory shows that for the highest efficiency the particles should be of a much smaller diameter than those in current use, but a significant move in this direction is constrained by the pressure required from the pumping system used to deliver the mobile phase. Capillary electrochromatography (CEC) uses an electric field rather than hydraulic pressure to drive the mobile phase through the packed bed of stationary phase. Since the resulting flow profile is plug-like rather than parabolic, very high efficiencies can be achieved.

Theory

The ability to drive a liquid through a packed capillary under the influence of an electric field was first described by Pretorius in 1974, but a lack of the necessary hardware at the time meant that his suggestion was not followed up. Pretorius showed that the linear velocity of a liquid (u) under electrical flow conditions was given by the equation:

$$u = \frac{\varepsilon E \zeta}{4\pi\eta} \qquad [1]$$

where E is the applied electrical field in V cm^{-1}; ε is the dielectric constant (dimensionless); ζ is the zeta potential in volts; and η is the viscosity of the liquid in kg m^{-1} s^{-1}.

Pretorius estimated from eqn [1] that, with voltages up to 1500 V cm^{-1}, it should be possible to generate linear velocities of 0.1 to 1 cm s^{-1}, which are similar to those used in modern HPLC. He also noted that eqn [1] is independent of particle diameter, which means that the flow rate could be maintained in a column packed with very fine material. When the various contributions to column plate height are summed and plotted against linear velocity, it can be seen that the overall dispersion in an electrically driven system is approximately half that of a corresponding pressure-driven system. This is almost entirely due to a dramatic decrease in the contribution of the eddy diffusion term in the electrically driven system, as illustrated in **Figure 1.**

The driving force in electrochromatography results from the electrical double layer that exists at any liquid–solid interface. The electrical double layer inside a fused silica capillary filled with an electrolyte is shown in **Figure 2.**

Under alkaline conditions, the surface silanol groups of the fused silica become ionized, leading to a negatively charged surface. This surface will have a layer of positively charged ions in close proximity that is relatively immobile. This surface layer of ions is called the Stern layer. The remainder of the excess charge, constituting the Goüy layer, is solvated and has the characteristics of a typical solvated ion. This layer extends into the bulk of the liquid and is the so-called double layer. The concentration of ions in the double layer is relatively small compared to the total ion concentration and falls off exponentially from the capillary surface, as does the electrical potential, which is proportional to the charge density. The potential at the boundary between the Stern layer and the diffused Goüy double layer is known as the zeta potential, ζ, and ranges from 0 to 100 mV. As the charge density drops off with distance from the surface, so does the zeta potential. The distance from the immobile layer to a point in the bulk liquid at which the potential is 0.37 of that at the interface between the Stern layer and the diffuse layer is defined as the thickness of the double layer, denoted by δ. The